Abscisic Acid Induces Formation of Floating Leaves in the Heterophyllous Aquatic Angiosperm *Potamogeton nodosus*

Abstract. Potamogeton nodosus *tubers produce floating-type instead of submersed-type leaves when exposed to 10^{-5} molar synthetic abscisic acid. Abscisic acid–induced leaves have stomata on upper leaf surfaces and higher width/length ratios than controls. These effects are wholly or partially overcome by simultaneous exposure to abscisic acid combined with gibberellic acid, kinetin, or benzyladenine.*

Potamogeton nodosus Poir. (American pondweed) is of agricultural and economic importance because of its interference with conveyance of irrigation water in many of the canal systems in the western United States. This species is like many heterophyllous aquatic angiosperms in that it produces two distinct types of leaves: long, narrow submersed leaves and ovate or elliptical floating leaves (*1*). The floating leaves have physical characteristics of terrestrial leaves: thick waxy cuticle, stomata on the upper (adaxial) surface, palisade mesophyll layer, and spongy mesophyll layer. In addition, large air spaces (lacunae) are present. Submersed leaves, which in this species are generally produced before floating leaves, are thin (three or four cells thick), have no stomata or heavy cuticle layer, lack large air spaces, and do not show any significant differences between "upper" and "lower" surfaces (Fig. 1).

The physiological mechanisms controlling the production of different leaf types in heterophyllous hydrophytes are not well understood, although some evidence suggests a role for normal developmental sequences or "heteroblastic" development (*2*), photoperiod (*3*), nutritional requirements (*4*), external osmotic conditions (*5*), and dehydration and hormonal effects (*6*). I have found that the plant growth regulator abscisic acid (ABA) induces the formation of floating leaves in totally submersed germinating tubers of *P. nodosus*, either at low concentrations (10^{-5} to $10^{-6}M$) or on recovery of vegetative tubers in which germination was initially inhibited by higher concentrations of ABA (2×10^{-5} to $5 \times 10^{-5}M$). Combinations of other plant growth regulators with ABA variously mitigated the ABA-induced formation of floating leaves, but none alone induced their formation. I believe this to be the first report of a specific plant growth regulator that causes the production of floating leaves and inhibits the formation of submersed leaves in a heterophyllous aquatic angiosperm.

Vernalized tubers of *P. nodosus* were germinated under controlled conditions (*7*) in 1/100 strength Hoagland's nutrient medium (*8*) alone or containing synthetic ABA, gibberellic acid (GA_3), benzyladenine (BA), kinetin (KT), or combinations of ABA and these plant growth regulators (*9*). The percentages of successful germinations and general growth and development were observed after 14 days. Abscisic acid alone inhibited germination completely at or above $5 \times 10^{-5}M$, partially at $2 \times 10^{-5}M$, and not at all at $10^{-5}M$. This inhibition was wholly or partially overcome by concomitant exposure to optimal concentrations of GA_3, BA, or KT. However, inhibition caused by ABA was not permanent, since ungerminated tubers germinated within 14 days after transfer to fresh medium not containing ABA. Plants from recovered tubers differed morphologically from those from untreated tubers in that the leaves were almost exclusively of the floating type. Microscopic examination of the upper and lower surfaces of the ABA-induced floating leaves revealed no differences from normal floating leaves (Fig. 1). Stomata were abundant on the upper leaf surface and rare or absent on the lower surface. The upper surface was waxy and appeared identical to that of normally produced floating leaves.

The observed changes in leaf morphology were quantified by two methods: leaf width/length ratio, and presence or absence of stomata (percentage of leaves

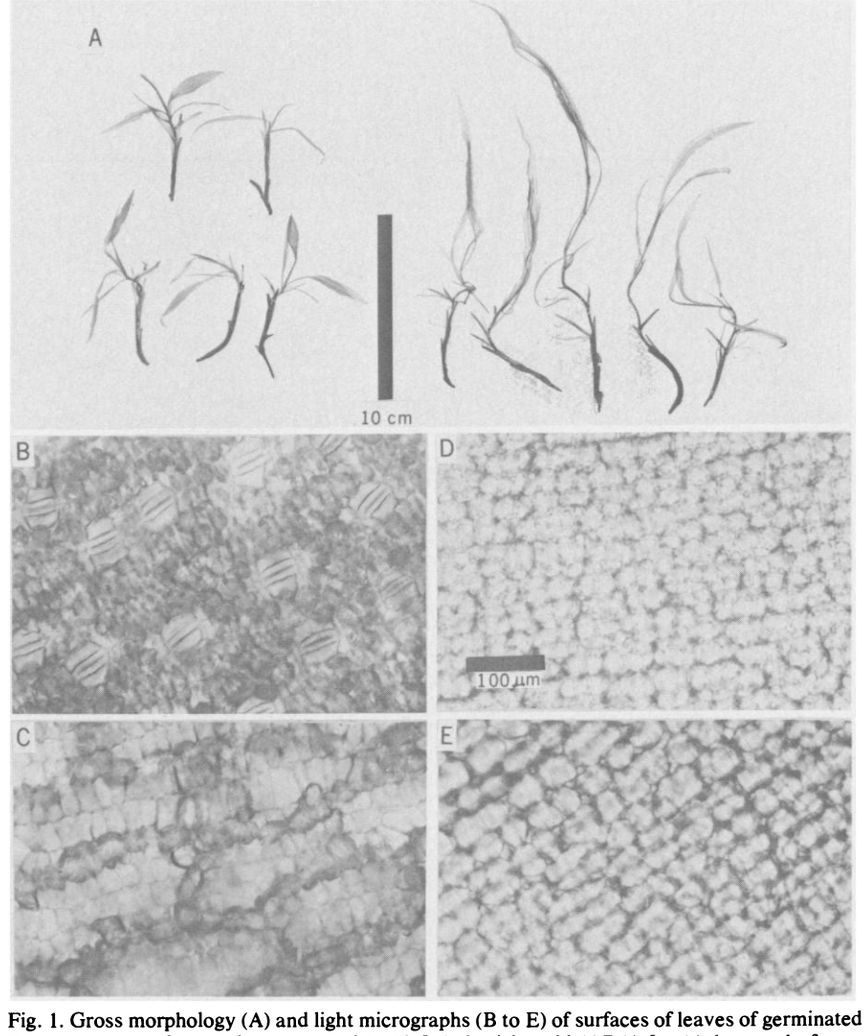

Fig. 1. Gross morphology (A) and light micrographs (B to E) of surfaces of leaves of germinated *Potamogeton nodosus* tubers exposed to $10^{-5}M$ abscisic acid (ABA) for 14 days and of controls. (A to E) Pictures on the left are of floating-type leaves and those on the right are of submersed-type leaves. (B and C) Upper and lower leaf surface, respectively, of ABA-treated plants. (D and E) Upper and lower leaf surface, respectively, of control plants. Note the presence of stomata on the upper leaf surface of ABA-treated plants (B). Floating-type leaves produced during the normal development of untreated plants appear identical to (B) and (C).

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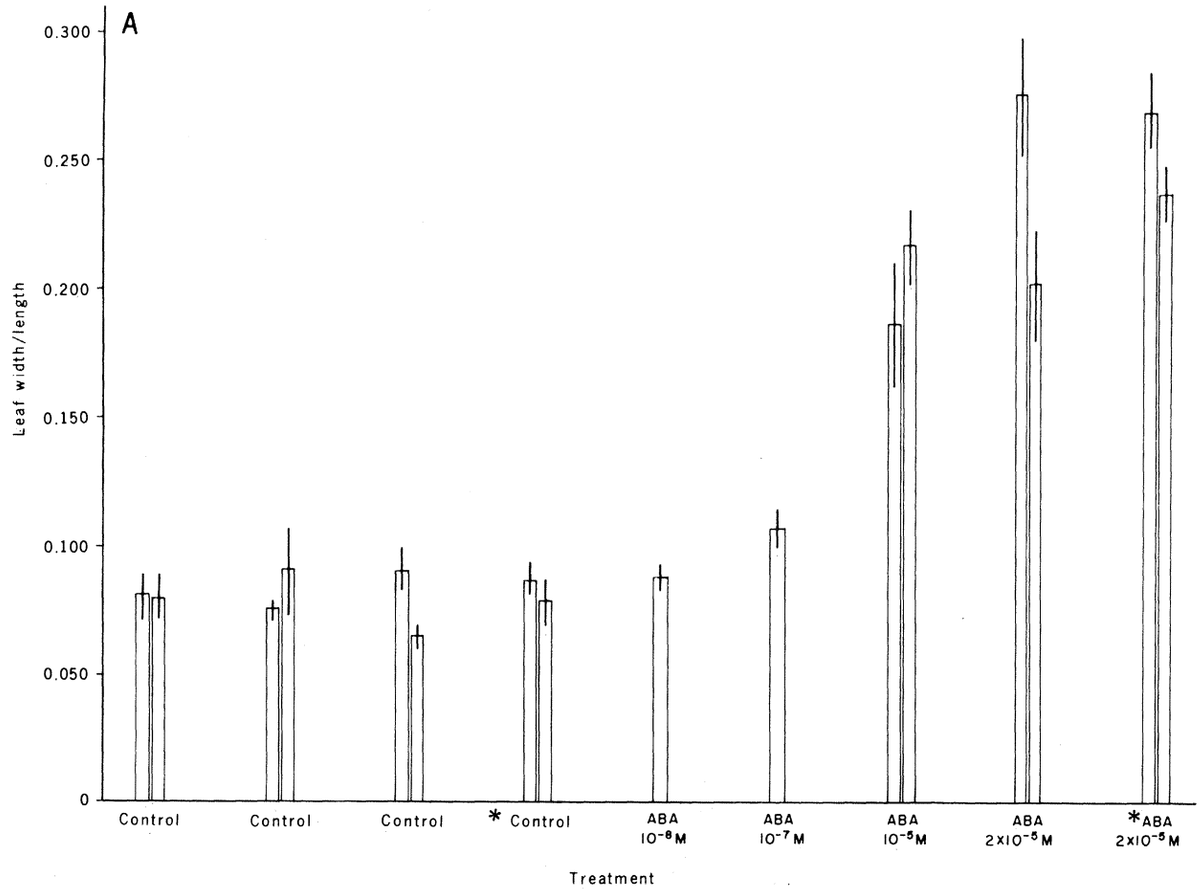
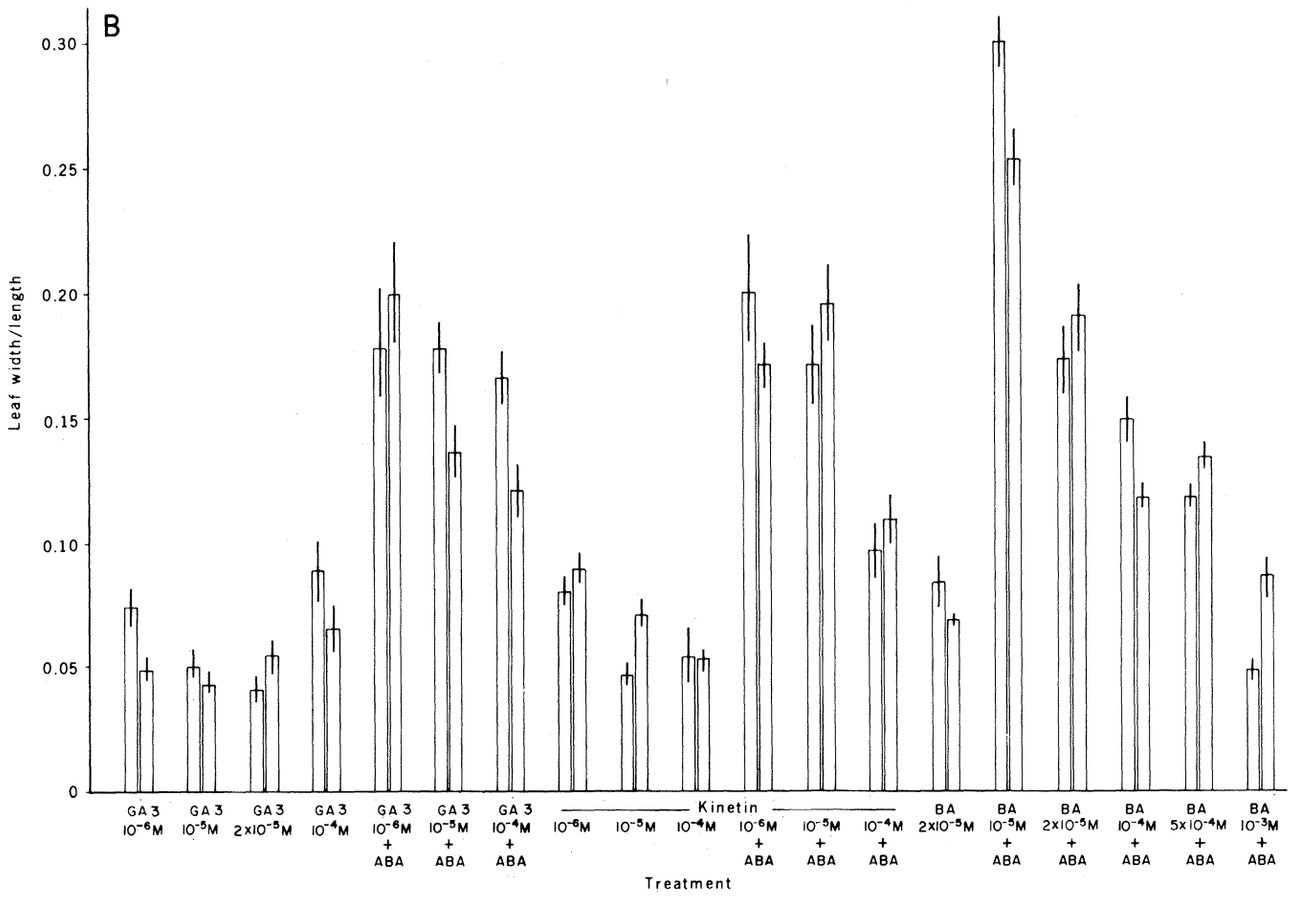

A
Leaf width/length
0.300
0.250
0.200
0.150
0.100
0.050
0
Control
Control
Control
* Control
ABA 10⁻⁸M
ABA 10⁻⁷M
ABA 10⁻⁵M
ABA 2x10⁻⁵M
*ABA 2x10⁻⁵M
Treatment


B
Leaf width/length
0.30
0.25
0.20
0.15
0.10
0.05
0
GA3 10⁻⁶M
GA3 10⁻⁵M
GA3 2x10⁻⁵M
GA3 10⁻⁴M
GA3 10⁻⁶M + ABA
GA3 10⁻⁵M + ABA
GA3 10⁻⁴M + ABA
Kinetin
10⁻⁶M
10⁻⁵M
10⁻⁴M
10⁻⁶M + ABA
10⁻⁵M + ABA
10⁻⁴M + ABA
BA 2x10⁻⁵M
BA 10⁻⁵M + ABA
BA 2x10⁻⁵M + ABA
BA 10⁻⁴M + ABA
BA 5x10⁻⁴M + ABA
BA 10⁻³M + ABA
Treatment

with stomata). Observations were made on germinating tubers that had been exposed to noninhibitory concentrations of ABA (10^{-6} to $10^{-5}M$), tubers allowed to recover from inhibitory concentrations of ABA, or tubers that had been exposed to $10^{-5}M$ ABA beginning 7 days after germination and continuing for 2 weeks thereafter. Leaf measurements were also made after exposure to combinations of ABA with GA_3, BA, or KT. The results are shown in Fig. 2. It is clear that only ABA (of the plant hormones used) caused the production of leaves with high width/length ratios, almost all of which had floating leaf characteristics. The ABA effect was partially overcome by appropriate concentrations of GA_3, BA, or KT.

Increasing concentrations of BA, GA_3, or KT in combination with ABA caused a graded reduction in the leaf width/length ratio. Benzyladenine was least effective, and GA_3 and KT were about equally potent, reducing the width/length ratio at around $10^{-5}M$ in the presence of $2 \times 10^{-5}M$ ABA. However, the counteracting effects of BA, GA_3, and KT on the production of stomata appear to be transient. Thus, although fewer leaves had stomata after exposure to ABA plus the plant growth regulators at $10^{-5}M$ for 2 weeks, after recovering in medium without plant growth regulators for an additional 2 weeks, most leaves had developed stomata (Fig. 2C). Similar results were obtained at $10^{-4}M$ GA_3 or KT, but BA continued to exert a mitigating effect on the ABA-induced production of stomata. It should be noted that the number of stomata per unit leaf area was not ascertained, only their presence or absence. A more graded response might have been obtained if the number of stomata per square millimeter had been determined. The results obtained on exposure to ABA after germination indicate that the effect is not dependent on the presence of ABA during initiation of germination (Fig. 2A).

The observed inhibition of tuber germination by ABA is consistent with reports implicating ABA in the regulation of dormancy in terrestrial plants (*10*). Weber and Nooden (*11*) also found that ABA inhibits germination of vegetative propagules (turions) in the aquatic angiosperm *Myriophyllum spicatum*, and that the level of endogenous ABA varies seasonally. Others have demonstrated that GA_3 causes a decrease in the leaf width/length ratio in aquatic and terrestrial plants (*6*, *12*), produces changes in phyllotaxis in *Xanthium pennsylvanicum* (*13*), suppresses the formation of terrestrial-type leaves in the aquatic fern *Marsilea drummondi* (*14*), induces the formation of submersed-type shoots in *Callitriche stagnalis* (*6*), and increases stem growth and lateral bud formation in the aquatic angiosperm *Ceratophyllum demersum* (*15*).

The significance of the present report is the demonstration of a novel plant response to ABA that results in heterophylly by shifting the normal differentiation of newly formed leaves from the submersed to the floating type. This is not merely an alteration in the relative activities of meristematic regions of the leaf primordia to produce a broader leaf; rather, ABA causes differentiation of the upper and lower leaf surfaces, leading to the more complex floating leaf type. Whether ABA acts directly on leaf differentiation or only accelerates normal ontogeny in *P. nodosus* is not clear. Abscisic acid has been shown to cause "senescence" responses in some terrestrial plants (*16*), and it may function similarly here. However, I have not observed flowering in plants allowed to grow up to 4 months after exposure to ABA. Per-

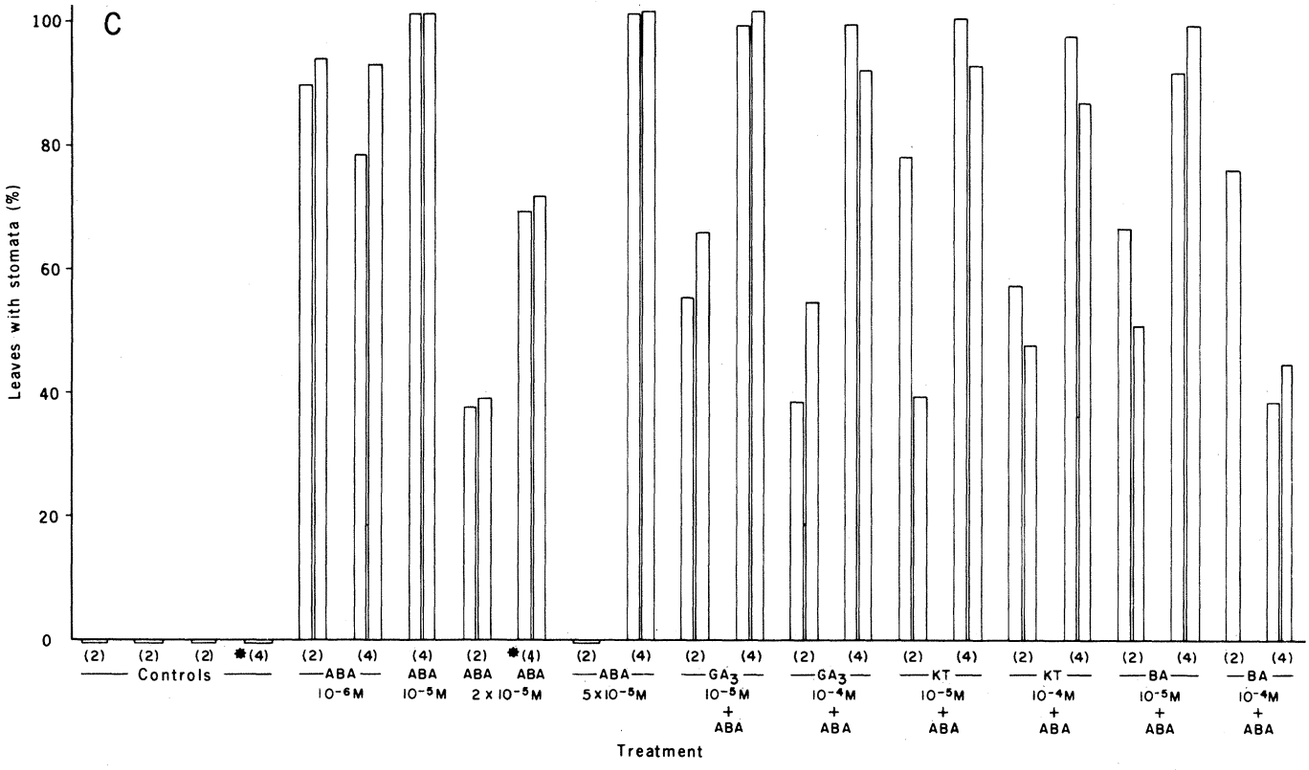


Fig. 2. Quantification of ABA-induced heterophylly in germinated tubers of *Potamogeton nodosus*. Data are duplicate (paired bars) means of 14-day treatments with ABA, gibberellic acid (GA_3), benzyladenine (*BA*), or kinetin (*KT*) alone, or in combinations of $2 \times 10^{-5}M$ ABA and one of the plant growth regulators. (A and B) Leaf width/length ratios and standard errors (vertical lines). In (A), controls from three separate experiments (first three bars) are shown to demonstrate normal variability in leaf width/length ratio. (C) Occurrence of stomata on leaf surface, means of three or four leaves per plant, five plants per replicate. Numbers in parentheses are observation times, weeks after incubation began. Asterisks indicate plants exposed to ABA 7 days after initiation of incubation, and corresponding untreated controls.

haps the most similar reported effect is the promotion of citrus bud callus formation by ABA (*17*).

The reported increases in endogenous levels of ABA in water-stressed terrestrial plants (*18*) and in the aerial rosettes of the aquatic angiosperm *C. stagnalis* (*19*) suggest interesting theories of the normal development of heterophylly in *P. nodosus*. In the natural habitat, the vegetative tubers usually germinate when completely underwater and the plant does not produce floating leaves until the submersed-type leaves have reached the surface. It is possible that desiccation of the uppermost surfaces of those submersed leaves when they reach the surface initiates production (or accumulation) of ABA, which in turn promotes the development of floating leaves at the apical meristems. This would also account for the observation that floating leaves are first produced in germlings at the aerially exposed, damp margins of ponds.

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References and Notes

1. For a review of heterophylly in aquatic plants, see A. Allsopp, *Handb. Pflanzenphysiol.* **15**, 1172 (1965); C. P. Sculthorpe, *The Biology of Aquatic Vascular Plants* (Arnold, London, 1967), pp. 218–247; G. E. Hutchinson, *A Treatise on Limnology* (Wiley, New York, 1975), vol. 3, pp. 157–196.
2. J. W. Wooten, *J. Ecol.* **58**, 232 (1970); G. Turesson, *Bot. Not.* **114**, 435 (1961).
3. J. M. Bostrack and W. F. Millington, *Bull. Torrey Bot. Club* **89**, 387 (1962); G. J. Davis, thesis, University of North Carolina (1956).
4. W. H. Pearsall and A. M. Hanby, *New Phytol.* **24**, 112 (1925); A. Allsopp, *Ann. Bot. (London)* **17**, 447 (1953); *Nature (London)* **173**, 1032 (1954); *Ann. Bot. (London)* **18**, 449 (1954); *J. Linn. Soc. London Bot.* **58**, 417 (1963); *Handb. Pflanzenphysiol.* **15**, 1236 (19→ R. A. White, *Am. J. Bot.* **53**, 158 (1966).
5. H. Jones, *Ann. Bot. (London)* **19**, 93 (1955); in *The Growth of Leaves*, F. L. Milthorpe, Ed. (Butterworth, London, 1956), p. 93; M. E. McCully and H. M. Dale, *Can. J. Bot.* **39**, 1099 (1961).
6. A. J. McComb, *Ann. Bot. (London)* **29**, 445 (1965).
7. Five tubers (= one replicate) were rinsed with tap water, weighed, and placed in 500-ml Erlenmeyer flasks containing 250 ml of 1/100 strength Hoagland's medium, with or without ABA or ABA and GA_3, BA, or kinetin, and were maintained in a growth chamber at 22°C on a 12-hour light schedule (~ 5000 lux cool-white fluorescent light). To examine the possible effects of bacteria and fungi occurring on harvested tubers, similar hormone treatments were conducted with sterile Hoagland's medium and tubers were soaked for 10 minutes in 5 percent sodium hypochlorite for surface sterilization. Sterile filtered (0.22 μm) hormones were added after the autoclaved medium had cooled. Data presented are from experiments under sterile conditions. Each treatment was in duplicate or triplicate. Each experiment was repeated one to three times.
8. D. R. Hoagland and D. I. Arnon, *Calif. Agric. Exp. Stn. Circ. 347* (1950).
9. Synthetic abscisic acid (racemate, A grade, lot 700339, Calbiochem) was initially dissolved in methanol and then diluted with two volumes of distilled water. In methanol, the ABA had a maximum absorption wavelength of 245 nm and appeared as a single ultraviolet-absorbing spot when chromatographed on silica gel plates developed in a mixture of *n*-butanol, *n*-propanol, ammonia, and water (2 : 6 : 1 : 2). The other plant growth regulators were gibberellic acid (75 percent, Nutritional Biochemicals, lot 5764), N^6-benzyladenine (Nutritional Biochemicals, lot 8076), and kinetin (6-furfurylaminopurine, Nutritional Biochemicals, lot 1838).
10. C. F. Eagles and P. F. Wareing, *Physiol. Plant.* **17**, 697 (1964); D. Aspinall, L. G. Paleg, F. T. Addicott, *Aust. J. Biol.* **20**, 869 (1967); D. C. Sumner and J. L. Lyon, *Planta* **75**, 28 (1967).
→ J. A. Weber and L. D. Nooden, *Am. J. Bot.* **63**, 936 (1976); *Plant Cell Physiol.* **17**, 721 (1976).
12. P. A. Arnozis, O. H. Caso, D. H. Cogliatti, *Phyton (Buenos Aires)* **35**, 1 (1977).
13. R. Maksymowych and R. O. Erickson, *Science* **196**, 1201 (1977).
14. A. Allsopp, *Phytomorphology* **12**, 1 (1962).
15. E. Best and R. Soekarjo, *Physiol. Plant.* **38**, 249 (1976).
16. B. V. Milborrow, *Annu. Rev. Plant Physiol.* **25**, 259 (1974).
17. A. Altman and R. Goren, *Plant Physiol.* **47**, 844 (1971); *Physiol. Plant.* **32**, 55 (1974).
→ S. T. C. Wright, *Planta* **86**, 10 (1969); B. V. Milborrow and R. C. Noodle, *Biochem. J.* **119**, 727 (1970).
19. B. V. Milborrow and D. R. Robinson, *J. Exp. Bot.* **24**, 537 (1973).
20. I thank J. E. Boutwell for his assistance in measuring and observation of leaves of *P. nodosus*.

30 March 1978; revised 14 June 1978

Inhibition of Bone Formation During Space Flight

Abstract. *Parameters of bone formation and resorption were measured in rats orbited for 19.5 days aboard the Soviet Cosmos 782 biological satellite. The most striking effects were on bone formation. During flight, rats formed significantly less periosteal bone than did control rats on the ground. An arrest line at both the periosteum and the endosteum of flight animals suggests that a complete cessation of bone growth occurred. During a 26-day postflight period, the defect in bone formation was corrected. No significant changes in bone resorption were observed.*

Changes in calcium homeostasis present a potential problem during prolonged space flights. Significant decreases in bone density have been documented after space flight in humans by photon absorptiometric techniques (*1*) and in monkeys by x-ray densitometry (*2*). Microscopic examination of long bones of young Wistar rats after a 22-day space flight aboard the Soviet biological satellite Cosmos 605 suggested that bone growth was inhibited during flight but returned to normal by 27 days after flight (*3*). Metabolic studies of Skylab astronauts indicated that during flight there was a significant increase in urinary calcium (*4*), similar in degree to that observed during bed rest (*1*, *5*), but no change in hydroxyproline (*6*). Since mechanical forces imposed by muscle utilization and gravity influence bone turnover (*7*), prolonged recumbency or prolonged weightlessness with continuous hypercalciuria and decreased bone mass could ultimately result in osteoporosis. To further define bone changes during space flight, parameters including bone formation and resorption were measured in tetracycline-labeled rats orbited for 19.5 days aboard the Soviet Cosmos 782 biological satellite.

Specific pathogen-free, male Wistar

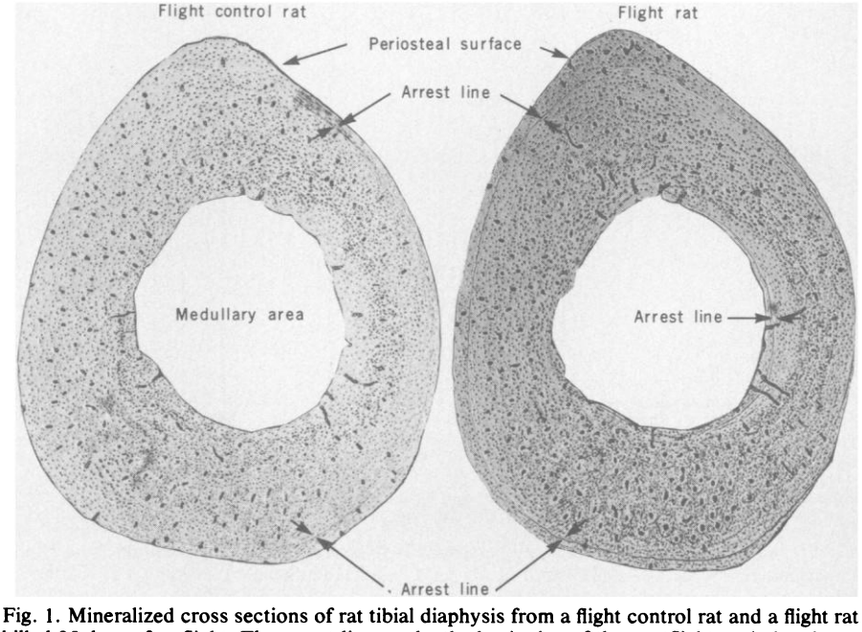

Fig. 1. Mineralized cross sections of rat tibial diaphysis from a flight control rat and a flight rat killed 25 days after flight. The arrest line marks the beginning of the postflight period and was not discernible in animals killed immediately after flight.

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